Correspondence to: Professor C K Fairley, School of Population Health, 2nd Floor, 723 Swanston Street, Carlton 3053, Australia; cfairley@unimelb.edu.au

References

1 **Hocking J**, Crofts N. HIV Surveillance in Victoria in 2000. Victorian Government Department of Human Services, Melbourne. *Victorian Infectious Diseases Bulletin* 2001;**4**:1–3.
2 **Fleming DT**, Wasserheit JN. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. *Sex Transm Infect* 1999;**75**:3–17..
3 **Anonymous**. HIV prevention through early detection and treatment of other sexually transmitted diseases—United States. Recommendations of the Advisory Committee for HIV and STD prevention. *Mor Mortal Wkly Rep CDC Surveill Summ* 1998;**47**(RR-12):1–24.

Accepted for publication 13 June 2002

Four layer discontinuous gradient for HIV

Artificial insemination using processed semen is a risk reduction option, if they want children, for serodiscordant couples in whom the man is HIV positive. The main aim of this study was to develop a single semen processing technique to reduce HIV transmission risks to HIV negative wives without infection and to obtain better quality sperm.

Methods

After ethics committee approval and written informed consent, normozoospermic semen was provided by two asymptomatic HIV carriers. Discontinuous four layer density gradient, whose fractions (Fr) were 1.065 (Fr 4), 1.085 (Fr 3), 1.110 (Fr 2), and 1.135 (Fr 1), was prepared with Puresperm. Semen washed with Hank's solution was laid on this gradient and centrifuged at 400 *g* for 30 minutes. The specimen of each fraction was extracted to determine sperm quality and to detect HIV RNA and proviral DNA using RT-PCR and PCR, respectively. Lymphocytes of an HIV non-carrier were co-cultured for 4 weeks with each fraction. HIV p24 antigen and proviral DNA after co-cultivation with each fraction were determined by indirect immunofluorescence assay and polymerase chain reaction (PCR), respectively.

Results

The percentage collection of sperm from Fr 1, Fr 2, Fr 3, and Fr 4 was 3% (SD 2%), 32% (9%), 19% (8%), and 10% (4%), respectively. Motility rate was 55% (19%), 94% (4%), 57% (25%), and 19% (11%), respectively. HIV proviral DNA and HIV RNA were detected only from Fr 4. HIV p24 antigen was observed in the lymphocytes co-cultivated with Fr 4 and from the positive control, but was not observed in other fractions. HIV proviral DNA was not detected from Fr 2 or Fr 3 (tables 1 and 2).

Table 2 Detection of HIV p24 antigen and proviral DNA after 4 weeks' co-cultivation with each fraction and carrier's PBL

	HIV p24 antigen	HIV DNA
Fr 1	neg	pos
Fr 2	neg	neg
Fr 3	neg	neg
Fr 4	pos	pos
Carrier's PBL	pos	pos

PBL = peripheral blood lymphocytes.

Discussion

HIV discordant couples have a risk of transmission generally if they wish to have a baby.[1 2] Semprini *et al*[3] reported continuous gradient centrifugation followed by a swim up procedure, and Marina *et al*[4] carried out a similar method but HIV was detected in 5.6% of 107 samples. However, the condition of the sperm, after these processes, was not always sufficient for intrauterine insemination.

We have developed a novel semen single processing technique to reduce HIV RNA and HIV proviral DNA to undetectable levels in the fraction whose sperm quality was higher than others. Furthermore, this fraction was confirmed to have no HIV infectivity in vitro. This method appears to be an attractive alternative for HIV discordant couples.

Contributors

KK and YA contributed to laboratory work; AY referred HIV positive volunteers.

K Kakimoto, Y Ando, A Yoshioka
International Medical Centre of Japan, Tokyo, Japan Correspondence to: K Kakimoto; kakimoto@sannet.ne.jp

References

1 **Saracco A**, Musicco M, Nicolosi A, *et al*. Man-to-woman sexual transmission of HIV: longitudinal study of 343 steady partner of infected men. *J AIDS* 1993;**6**:497–502.
2 **Lazzarin A**, Saracco A, Musicco M, *et al*. Man-to-woman sexual transmission of the human immunodeficiency virus. Risk factors related to sexual behavior, man's infectiousness, and woman's susceptibility. *Arch Intern Med* 1991;**151**:2411–6.
3 **Semprini AE**, Levi-Setti P, Bozzo M, *et al*. Insemination of HIV-negative women with processed semen of HIV-positive partners. *Lancet* 1992;**340**:1317–9.
4 **Marina S**, Marina F, Alcolea R, *et al*. Human immunodeficiency virus type 1 —serodiscordant couples can bear healthy children after undergoing intrauterine insemination. *Fertil Steril* 1998;**70**:35–9.

Accepted for publication 1 May 2002

Table 1 Sperm characteristics and detection of HIV in each fraction

	Sperm collection rate (%)	Sperm motility rate (%)	HIV RNA	HIV DNA	HIV p24 antigen after co-cultivation	HIV DNA after co-cultivation
Fr 1	3 (2)	55 (19)	negative	negative	negative	positive
Fr 2	32 (9)	94 (4)	negative	negative	negative	negative
Fr 3	19 (8)	57 (25)	negative	negative	negative	negative
Fr 4	10 (4)	19 (11)	positive	positive	positive	positive

Erythema nodosum induced by chancroid

Erythema nodosum is a type of panniculitis which is often regarded as a complex reaction pattern to various aetiological factors of infective and non-infective origin.[1] Infective agents outnumber inflammatory causes and drugs in causation of erythema nodosum in the developing countries. Almost all the infective agents including aerobic and anaerobic bacteria, viruses, fungi, parasites and mycobacteria can induce eruption of erythema nodosum.[2] Among sexually transmitted infections lymphogranuloma venereum has been known to be associated with erythema nodosum not infrequently.[3]

A 23 year old woman presented with genital ulcer disease and painful rash over the legs of 1 week's duration. There was no history of trauma, fever, or drug intake. She had a single stable sexual partner who was apparently unaffected. Examination revealed a single, 1–1.5 cm size, irregular tender ulcer on the right labia minora with undermined margins and bleeding on touch. The right inguinal lymph nodes were firm, moderately enlarged, and tender. Speculum and vaginal examination was normal. Examination of the perianal region, perineum, and other mucosae was also normal.

Multiple tender, erythematous nodular subcutaneous lesions with dusky erythema were present over both shins, calves, and ankle joints. Investigations revealed a normal complete blood count, serum biochemistry, urinalysis and blood sugar. VDRL, HIV-1 ELISA, and HBsAg were negative. Dark ground illumination, smears, and cultures from the ulcer did not reveal aetiological diagnosis. Histopathology from the ulcer revealed an ulcerated surface with necrosis and neutrophilic infiltrate deeper to which a zone of new blood vessel formation with marked endothelial proliferation and a lymphoplasmacytic infiltrate was observed. These features were consistent with diagnosis of chancroid while histopathology of leg lesions confirmed it to be septal panniculitis consistent with a diagnosis of erythema nodosum. The patient was treated with erythromycin stearate 500 mg 6 hourly for 7 days. The genital ulcer healed completely in 7–10 days but the lesions of erythema nodosum subsided completely in 5–7 days without any other treatment.

Erythema nodosum as a cutaneous reaction pattern was first observed by Willan in 1798.[4] A female preponderance with a ratio of 3:1 is often observed in adults compared to an equal incidence at prepubertal age. Although the exact pathogenesis of erythema nodosum is not known, it has been regarded as a immune complex, deposition disease which prefers the richly supplied vascular adipose tissue of the legs.

In the present patient erythema nodosum and chancroid had a strong temporal correlation as erythema nodosum immediately followed the appearance of the chancroid and resolved completely with its resolution. Although erythema nodosum is known to be associated with innumerable infective agents, to the best of our knowledge chancroid leading to causation of erythema nodosum has not been observed before.

C Kaur, G P Thami
Department of Dermatology and Venereology, Government Medical College Hospital, Sector 32 B, Chandigarh 160030, India

Correspondence to: Dr G P Thami; drgurvinder@mantraonline.com